The Veliger 26(3):199-206 (January 3, 1984)

THE VELIGER

Hybridization of Two Populations of a Marine Opisthobranch with Different Developmental Patterns

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Abstract. Two populations of the ascoglossan opisthobranch *Elysia chlorotica* (Gould, 1870) have different reproductive modes. In one population, from Martha's Vineyard, Massachusetts, veliger larvae hatched from egg masses and fed on phytoplankton prior to metamorphosis. In the second population, from Ipswich, Massachusetts, the majority of veligers completed development up to and including metamorphosis in egg capsules before hatching. The Ipswich population laid egg masses with fewer eggs, and both eggs and egg capsules were larger than those of the Martha's Vineyard population.

Both populations can be cultured in the laboratory. The Ipswich population was cultured both in dilute (17‰) and full strength (33‰) seawater. In laboratory culture, Ipswich larvae metamorphosed in the egg capsule without substrate, but metamorphosis of the planktonic veligers (Martha's Vineyard) required the alga *Vaucheria* sp.

Laboratory-reared individuals from the two populations hybridized. F_1 egg masses had either large Ipswich-type egg capsules or smaller Martha's Vineyard-type egg capsules. The majority of veligers from F_1 Ipswich-type capsules remained in capsules up to metamorphosis. All veligers from F_1 Martha's Vineyard-type capsules hatched prior to metamorphosis. In F_2 egg masses, capsule size was intermediate between the Ipswich and Martha's Vineyard size classes. All F_2 veligers hatched prior to metamorphosis and required *Vaucheria* sp. for metamorphosis.

INTRODUCTION

THOMPSON (1967, 1976) divided opisthobranch development into three distinct categories—planktotrophic, lecithotrophic, and direct development. Development in each category differs with respect to the size and number of eggs produced and the stage of development reached when veligers hatch from egg masses. In general, planktonic developers produce many small eggs which hatch prior to metamorphosis. Lecithotrophic developers produce fewer intermediate-sized eggs which hatch prior to metamorphosis but after a longer embryonic period than planktonic developers. Direct developers produce a much smaller number of large eggs and remain encapsulated up to metamorphosis.

BONAR (1978) has refined THOMPSON's scheme and defined two variations within the category of direct development. In some cases of direct development—referred to as ametamorphic capsular development—embryos do not develop into a distinct veliger stage and, therefore, do not undergo metamorphosis before hatching as juveniles. In other cases—referred to as capsular metamorphic development—embryos develop into a distinct veliger stage but do not hatch out of capsules prior to metamorphosis.

Most species of opisthobranch are limited to one of the

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reproductive patterns outlined by THOMPSON and refined by BONAR. However, there are reports of more than one reproductive pattern within a species (FRANZ, 1971; RIVEST, 1978; EYSTER, 1979; CLARK *et al.*, 1979). In only one case (EYSTER, 1979) was an attempt made to determine whether distinct differences in developmental pattern between members of a species were accompanied by reproductive isolation.

This report compares the different reproductive patterns in two populations of the euryhaline ascoglossan opisthobranch *Elysia chlorotica* (Gould, 1870). Planktotrophic development already has been described for the species (HARRIGAN & ALKON, 1978a). We describe here the capsular metamorphic type of direct development in a second population.

If this is a true case of developmental variability within a species (poecilogony), crosses between individuals from the different populations should produce viable hybrid offspring. It was the purpose of this study to determine whether the two populations could be cultured and hybridized and to compare the reproductive traits expressed in the field-collected, laboratory-reared, and hybrid generations.

MATERIALS AND METHODS

Culture of Algae

Successful culture of *Elysia chlorotica* depends upon the culture of appropriate algal food sources for both adult and larval stages. Adults feed on various species of the alga *Vaucheria*; the veligers require unicellular algae. Mats of substrate, containing filaments of *Vaucheria compacta* (Collins) Collins, were collected in salt marshes and placed in petri dishes moistened with seawater. The mats were kept in an incubator at 15°C on an 18-h light : 6-h dark cycle. Light was supplied by six fluorescent bulbs. A second species, *Vaucheria litorea* C. Agardh, also collected from salt marshes, was grown submerged in "f/2" algal medium of GUILLARD (1975) under the same conditions. The unicellular alga *Chroomonas* (strain 3C), used to feed the veligers, was cultured in "f/2" medium under the same conditions as *Vaucheria*.

Culture of Encapsulated Larvae

Approximately 100 adult animals collected from Ipswich, Massachusetts were placed in glass baking dishes containing seawater of salinity 33 parts per thousand (‰) which was changed every other day. The animals were kept in an incubator at 18–19°C on a 12-h light : 12-h dark cycle. Light was provided by four fluorescent bulbs. *Vaucheria compacta,* often attached to marsh sediment, was placed with the animals as a food source. Egg masses were collected daily and each egg mass was transferred to a small, glass petri dish containing seawater filtered through a Millipore membrane (0.45 μm pore). In order to classify the development type, the following observations and measurements were made: (1) the number of eggs per egg mass was determined in six egg masses by counting the number of eggs in 5 mm of egg mass, dividing by 5, and multiplying the result by the total length of egg mass; (2) the size of five uncleaved eggs and their capsules was measured using an ocular micrometer and compound light microscope; and (3) the number of days to veliger stage, eyespot formation, and metamorphosis was recorded. In addition, adult sizes were recorded as unrelaxed length using a millimeter rule.

Elysia chlorotica inhabits low salinity marshes. In the course of collecting slugs we measured salinities from 3 to 32‰. Because this is such a wide range of salinities, the effect of salinity on development was investigated. Adults from Ipswich, Massachusetts were kept in dilute seawater (17‰). Egg masses were collected from these adults and the pattern of development was observed to determine the effect salinity had on development.

Culture of Planktonic Veligers

Ten adults collected from Menemsha Pond, Martha's Vineyard, Massachusetts in the fall of 1979 survived over winter and laid egg masses in the spring of 1980. Egg masses were isolated and veligers maintained up to metamorphosis according to the general methods described by HARRIGAN & ALKON (1978a). Measurements of (1) the number of eggs per egg mass, (2) the size of eggs and egg capsules, and (3) the size of adults, were made as previously described. The time required for veligers to reach various stages of development was determined. Veligers were ready to hatch shortly after the development of a black pigment band on the dorsal surface behind the velum. At this stage, the egg masses were teased open, and the veligers released were washed in seawater filtered through Millipore membranes (0.22 μm pore) with Rifampicin added (5 mg/L). Approximately 1000 veligers were transferred to each of three 1-liter beakers containing 800 mL of filtered (0.22 μm) seawater containing Rifampicin (5 mg/L). Cetyl alcohol was sprinkled on the surface of the culture to prevent the veligers from being caught in the surface tension (HURST, 1967). Beakers were covered with plastic to minimize contamination by dust. Seawater in the beakers was changed three times a week. To separate veligers from culture water, cultures were gently poured through 44-μm plankton netting stretched across the diameter of a piece of plastic (PVC) pipe. The pipe was held upright in a finger bowl filled with seawater such that the plankton net and veligers were always below the surface of the water. Veligers concentrated above the plankton netting were washed with fresh seawater then transferred with a pipette to beakers of fresh seawater and Rifampicin.

The veligers were fed *Chroomonas* six days a week. After determining the cell count of algal cultures with a hemocytometer, a sufficient volume of algal cells was added

to the veliger cultures to yield a final cell count of 3 to 5 cells/μL.

The veligers were judged competent to metamorphose on the basis of three criteria: (1) development of eyespots; (2) development of a propodium; and (3) enlargement of the black pigment patch initially located immediately posterior to the velum over the entire dorsal surface of the larva. Once judged competent, groups of 50 veligers were placed in culture dishes containing 100 mL of coarse-filtered (1 μm) seawater and filaments of *Vaucheria*. Veligers attached to the algae and metamorphosed. Subsequently juveniles were transferred to larger culture dishes and eventually into aquaria. The juveniles were continuously fed *Vaucheria compacta* because it was the most abundant *Vaucheria* species. The number of veligers surviving up to metamorphosis and the number that subsequently metamorphosed were determined.

Metamorphosis on Different Algal Substrates

A separate experiment was performed to determine whether other substrates could be used for metamorphosis. One hundred veligers were placed in culture dishes with either *Vaucheria compacta, V. litorea, Enteromorpha* sp., or *Bryopsis plumosa* (Hudson) C. Agardh (all collected from Martha's Vineyard), and the number of metamorphosed juveniles was counted. *Enteromorpha* sp. was chosen because it represents a major part of the pond flora. *Bryopsis* is not common in the pond but one species of this genus has previously been identified as a food source for other ascoglossan species (Greene, 1970).

Laboratory Crosses—Parental, F_1, and F_2 Generations

Parents for the crosses were the offspring from egg masses laid by field-collected Ipswich and Martha's Vineyard adults. These offspring were raised according to the culture procedures described above except that all animals were raised at room temperature (25°C). Measurements of the characteristics of parental egg masses were made on the egg masses collected from crosses of the field-collected adults—Ipswich × Ipswich and Martha's Vineyard × Martha's Vineyard. Length of egg capsules, length of veliger shells, and the days to hatching after egg-mass deposition were recorded in 10 egg masses of each type. Directly following metamorphosis, 10 offspring from each developmental type were isolated. Each Ipswich offspring was placed with a Martha's Vineyard offspring to form a mating pair (Ipswich × Martha's Vineyard). Because the species is hermaphroditic, each individual in a cross could serve as both male and female. Egg masses from these parental mating pairs were the F_1 egg masses that gave rise to the F_1 adult generation.

Each F_1 egg mass was scored according to parental affinity. A Martha's Vineyard-type F_1 egg mass was defined as having all egg capsules of small length and veligers that hatched with no eyespots or propodium. An Ipswich-type egg mass had large capsules and veligers that either hatched with eyespots and propodium late in development or metamorphosed in capsules. Measurements of capsule length, veliger shell length, and days to hatching were made from 10 F_1 Martha's Vineyard-type egg masses and 10 F_1 Ipswich-type egg masses. Offspring from F_1 Martha's Vineyard-type egg masses and F_1 Ipswich-type egg masses were isolated directly following metamorphosis. Ten mating pairs were formed by placing one offspring from a Martha's Vineyard-type egg mass with one offspring from an Ipswich-type egg mass (Ipswich F_1 × Martha's Vineyard F_1). Egg masses from these F_1 crosses were the F_2 egg masses that gave rise to the F_2 generation. Measurements of capsule length, veliger shell length, and days to hatching were made on 10 of these F_2 egg masses.

To determine whether the slugs self-fertilized, newly metamorphosed offspring from parental egg masses of each developmental type were isolated and observed for possible egg mass deposition. In addition, some newly metamorphosed individuals from parental egg masses of each developmental type were grouped together (Ipswich × Ipswich and Martha's Vineyard × Martha's Vineyard). The F_1 and F_2 generations produced by these intrapopulation crosses served as controls to monitor the effects of laboratory culture on developmental type.

RESULTS

Culture of Encapsulated Larvae

Developmental characteristics of the population from Ipswich, Massachusetts are listed in Table 1. Field collected animals had a mean length of 7.60 mm. The mean number of eggs/egg mass was 175.67. A distinctive feature of this development type was the relatively large diameter of the egg capsule in relation to egg diameter. The mean capsule length of 309.00 μm was three times the mean diameter of the egg (96.00 μm). In addition, the capsules and embryos were surrounded by a thick gelatinous layer. There was a statistically significant decrease in development time for egg masses raised in dilute seawater (Table 2). At both salinities the embryos reached the veliger stage in approximately three days. All veligers eventually developed black pigment bands on the dorsal surface of the velum. Eyespots appeared in larvae approximately six days after egg mass deposition in dilute seawater and nine days after deposition in full strength seawater. About two weeks were required for eggs in full strength seawater to develop through metamorphosis.

Usually the encapsulated veligers would cast off the shell inside the capsule and crawl away as metamorphosed juveniles. However, some veligers from egg masses in full strength and dilute seawater hatched before metamorphosis while others in the same egg mass remained two to three days longer in the capsule and metamorphosed before hatching. We were not able to follow the fate of the

Table 1

Developmental features of two *Elysia chlorotica* populations. All values expressed as mean ± SD, followed by sample size in parenthesis. * Significant at the 0.01 level; ** 20 veligers observed in one culture only, in all other cases embryos were observed in more than one egg case.

	Ipswich population (encapsulated metamorphosis)	Martha's Vineyard population (planktonic)	*t*-statistic
Length of field-collected animals	7.60 ± 2.58 mm (133)	20.01 ± 8.00 mm (233)	*17.56
Eggs per egg mass	175.67 ± 112.53 (6)	8901.5 ± 7257.9 (16)	*2.68
Diameter of egg	96.00 ± 8.22 μm (5)	79.33 ± 2.58 μm (15)	*7.18
Length of egg capsule	309.00 ± 8.22 μm (5)	164.00 ± 12.42 μm (15)	*24.17
Days to veliger after deposition	3.00 ± 0 (93)	2.88 ± 0.32 (34)	*3.49
Days to eyespot after deposition	9.00 ± 0 (93)	**13–15 (20)	—
Days to metamorphosis after deposition	13.83 ± 8.60 (93)	21.56 ± 0.50 (34)	*49.18

early hatching veligers. After hatching from the egg masses, juveniles would begin to feed on *Vaucheria* filaments and the previously clear digestive gland turned a dark green color.

Culture of Planktonic Veligers

Developmental characteristics of the population from Martha's Vineyard are also listed in Table 1. Field-collected animals had a mean length of 20.01 mm. The mean number of eggs/egg mass was 8901.5. The mean egg diameter was 79.33 μm or about half the mean capsule length, which was 164.00 μm. The egg masses lacked the thick gelatinous layer of the encapsulated metamorphosis type. Embryos reached the veliger stage in approximately three days and developed dorsal black pigment bands before hatching. After hatching on day 7 or 8 following egg mass deposition, veligers spent 14 to 15 days feeding on unicellular algae prior to metamorphosis. Eyespots developed 13 to 15 days after egg mass deposition. As the veligers matured a propodium developed, and just prior to metamorphosis the black pigment band spread to cover much of the dorsal surface of the animal. When the pigmented veligers were presented with filaments of *Vaucheria,* they settled on the algal filament, velum down, with the shell lifted upwards. Metamorphosis took place over a period of one to two days. Occasionally, metamorphosis occurred spontaneously in culture beakers before the veligers were exposed to the algal substrate.

Metamorphosis on Different Algal Substrates

Of the 100 veligers placed in each of the culture dishes with the different algal species, 50 to 60 metamorphosed in the dishes containing either *Vaucheria compacta* or *V. litorea.* Only one metamorphosed juvenile was found in either of the culture dishes containing *Enteromorpha* or *Bryopsis.*

Statistical Comparison of the Two Populations

The results of the two-tailed "Student's" *t*-tests comparing the developmental characteristics of the two populations are listed in Table 1. For all developmental characteristics compared there was a statistically significant difference between the two populations ($P \leq 0.01$).

Laboratory Crosses—Parental, F_1, and F_2 Generations

Egg capsule length and shell length at hatching: Figure 1 shows the distribution of egg capsule lengths and shell lengths at hatching measured from egg masses deposited by the parental, F_1, and F_2 generations. The F_1 generation consisted of a total of 25 morphologically Ipswich-type egg masses (Ipswich F_1) and 34 morphologically Martha's Vineyard-type egg masses (Martha's Vineyard F_1). Figure 1 illustrates that both capsule length and shell length of the F_1 egg masses were distributed bimodally; each set of measurements corresponded closely to those of the similar parental generation.

Table 2

Developmental features of *Elysia chlorotica* (Ipswich population) at two salinities. All values expressed as mean ± SD, followed by sample size in parenthesis. * Significant at the 0.01 level.

	Salinity at 33‰	Salinity at 17‰	*t*-statistic
Days to veliger after deposition	3.00 ± 0 (93)	2.33 ± 0.49 (12)	*13.51
Days to eyespot after deposition	9.00 ± 0 (93)	5.92 ± 0.29 (12)	*106.55
Days to metamorphosis after deposition	13.83 ± 0.86 (93)	9.25 ± 0.87 (12)	*17.21

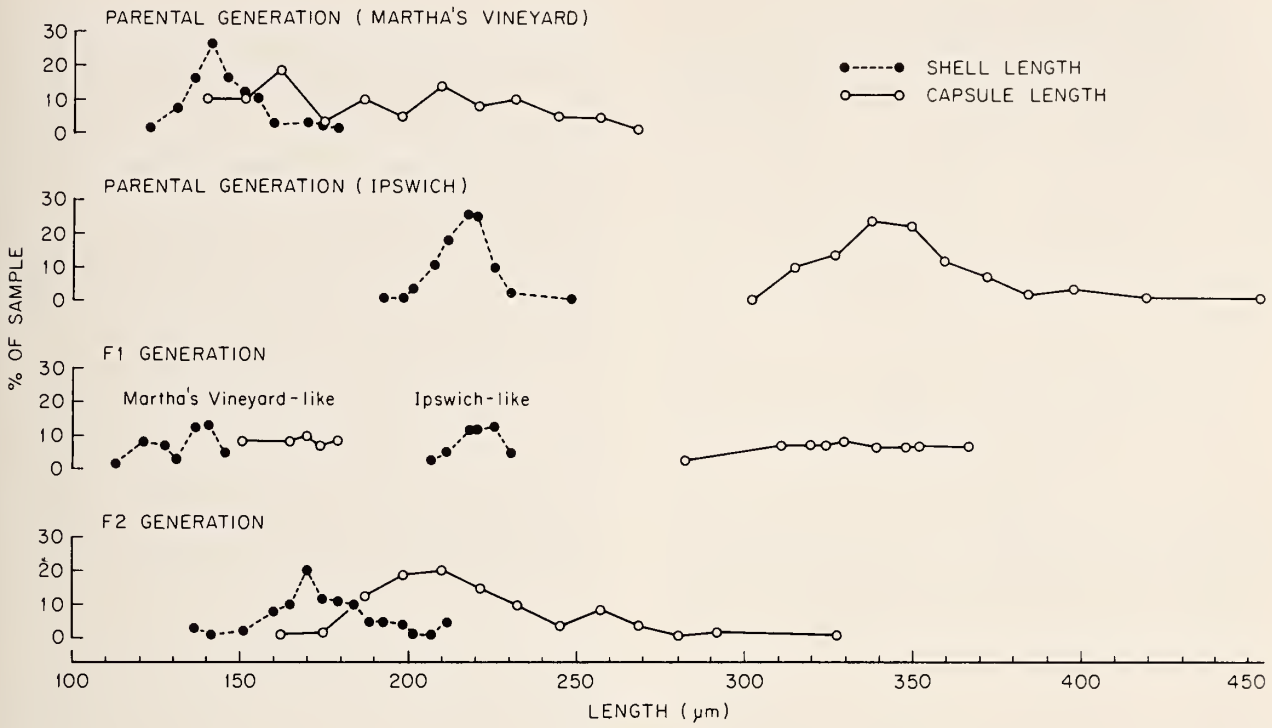

Figure 1

Relationship between shell length (μm) at hatching and egg capsule length (μm) for each population. Note especially the bimodal distributions of shell and capsule lengths in the F_1 generation and convergence of these characters in the F_2 generation.

In contrast to the F_1 egg masses, the 48 F_2 egg masses could not be classified as either Ipswich-type or Martha's Vineyard-type. Both capsule length and shell length at hatching are distributed unimodally (Figure 1), with the means falling between those of the parental and F_1 generations (Table 3). In all generations, egg capsule sizes were more variable than shell lengths.

Egg capsule lengths and shell lengths for all five populations combined were highly significantly different when analyzed using one-way ANOVA for unequal sample sizes (Snedecor & Cochran, 1967) ($F_{4,355} = 3027.3$, $P \leq 0.01$ for capsules; $F_{4,355} = 200.4$, $P \leq 0.01$ for shells). Subsequent application of the Newman-Keuls modification of the Q-test for significant differences between means showed that F_2 capsule lengths and shell lengths differed significantly from those of both the Martha's Vineyard F_1 and Ipswich F_1 populations ($D = 11.1$ μm for shells, $P \leq 0.05$; $D = 20.9$ μm for capsules, $P \leq 0.05$; and Figure 2). The Martha's Vineyard F_1 and parental generations also differed significantly, but the Ipswich F_1 and the Ipswich parental generations did not (Figure 2).

Hatching and metamorphosis: Table 4 lists the developmental stage at hatching and the time from egg mass

Table 3

Laboratory crosses of *Elysia chlorotica*: egg capsule length and shell length per generation. All values expressed as mean ± SD, followed by sample size in parenthesis.

Generation	Capsule length (μm)	Shell length (μm)
Parental—Martha's Vineyard	192.1 ± 36.3 (100)	145.8 ± 10.6 (80)
Parental—Ipswich	348.8 ± 24.3 (100)	216.7 ± 7.9 (100)
F_1—Martha's Vineyard-type	167.2 ± 10.2 (30)	134.1 ± 8.7 (30)
F_1—Ipswich-type	330.6 ± 23.8 (30)	220.6 ± 6.6 (30)
F_2	219.3 ± 28.9 (100)	176.5 ± 16.2 (100)

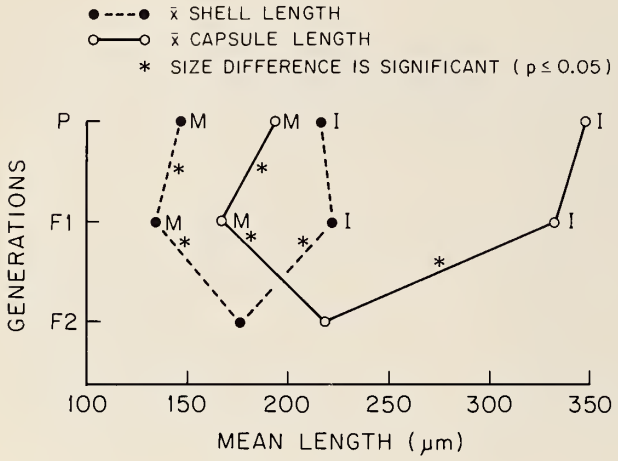

Figure 2

Mean shell and capsule lengths per generation; asterisk indicates means significantly different at $P \leq 0.05$. P, parental generation; F_1, first generation; F_2, second generation; M, Martha's Vineyard (P) or Martha's Vineyard-like (F_1) population; I, Ipswich (P) or Ipswich-like (F_1) population.

deposition to hatching for each population. The trend in the crosses is toward reduction of time spent in intracapsular development and the production of planktotrophic veligers. Although the F_2 veligers are significantly larger than the Martha's Vineyard parental veligers (Table 3 and Figure 2), they hatch in about the same amount of time—five to seven days for the Martha's Vineyard generation *versus* five to nine days for the F_2 generation (Table 4). The F_2 veligers also differ from the Martha's Vineyard parental veligers because eyespots are found in a variable fraction of the hatchlings from each egg mass.

The days from egg mass deposition to hatching were significantly different overall for the F_1 and F_2 populations ($F_{2,57} = 105.6$, $P \leq 0.01$). By the Newman-Keuls Q-test, the number of days to hatching in the F_2 generation, which appears intermediate in time between the Martha's Vineyard and Ipswich F_1 populations, differed significantly from days to hatching of both the Martha's Vineyard-type F_1 and Ipswich-type F_1 populations ($D = 0.2$ days, $P \leq 0.05$; and Table 4).

Self-fertilization and control crosses: Some of the newly metamorphosed individuals that were isolated from parental egg masses did lay egg masses. However, development of eggs in these egg masses was abnormal in both populations and no juveniles were produced.

All F_1 and F_2 egg masses produced by intrapopulation crosses (Ipswich × Ipswich and Martha's Vineyard × Martha's Vineyard) were of the parental developmental type. That is, F_2 egg masses laid in the Ipswich crosses had large capsules and most juveniles hatched from egg capsules following metamorphosis; F_2 egg masses laid by the Martha's Vineyard crosses had small capsules and hatched as veligers without eyespots.

DISCUSSION

Compared to other opisthobranchs, *Elysia chlorotica* is cultured with relative ease. First, the culture of direct developers is simple compared to planktonic developers because they do not require unicellular algae for growth and special techniques to change culture water. Also, in this study, normal development of direct developers took place even with a significant change in salinity.

Although it is more difficult to feed and clean them, the planktotrophic veligers of this species have a short planktonic stage when compared with 34 to 40 days in other opisthobranchs (Switzer-Dunlap & Hadfield, 1977; Harrigan & Alkon, 1978b; Chia & Koss, 1978). The key to the successful culture of the planktonic veligers is the identification of the substrate for metamorphosis, in

Table 4

Laboratory crosses of *Elysia chlorotica*: developmental stage at hatching and time to hatching in each generation.

Generation	Egg masses/ generation	Hatching stage	Days to hatching after deposition (mean ± SD)
Parental—Martha's Vineyard	10	100% veligers (no eyespots, no propodium)	5.8 ± 1.0
Parental—Ipswich	10	100% juveniles	10.67 ± 0.5
F_1—Martha's Vineyard-type	34	100% veligers (no eyespots, no propodium)	5.2 ± 0.8
F_1—Ipswich-type	25	27% veligers with eyespots and propodium; 59% mixed veligers and juveniles; 17% juveniles only	10.2 ± 2.0
F_2	48	100% veligers, some with eyespots and propodium	7.0 ± 0.8

this case, *Vaucheria*. It is not surprising that *Vaucheria* is the substrate for metamorphosis because adult digestive cells contain symbiotic chloroplasts which originate from *Vaucheria* (GRAVES *et al.*, 1979; WEST, 1979).

There are two important differences between direct development and planktotrophic development among opisthobranch species. First, in the direct development of any species, veligers require a stored food source because they do not feed on unicellular algae. Food reserves are stored as yolk in the egg. Consequently, opisthobranchs with direct development usually have large eggs, ranging from 205 to 400 μm in diameter (THOMPSON, 1967, 1976). Although the population with direct development in this study has a larger egg size than the population with planktotrophic veligers, the average diameter is only 96 μm. It is possible that food reserves may be stored in extraembryonic albumen in addition to yolk, which could account for the large capsule size in the direct developers. CLARK & JENSEN (1981) have proposed that large capsules and extraembryonic nutrients are typical of direct development in all ascoglossans.

A second difference accompanying direct development is that encapsulated metamorphosis takes place without external cues (BONAR, 1978). Models of metamorphosis of planktonic larvae involve some precise chemical cue which triggers a neuronal response in mature veligers (HADFIELD, 1978). Differences between Ipswich and Martha's Vineyard veligers could present an interesting problem in developmental neurobiology. It may be significant that both the present study and the study by HARRIGAN & ALKON (1978a) found that not all Martha's Vineyard veligers required *Vaucheria* for metamorphosis. If planktonic development was the original development type of the species, it could be that populations with encapsulated metamorphosis developed from the certain percentage of all veligers which could metamorphose without external cue.

There are two other species of opisthobranch where the existence of more than one developmental pattern in the species is described in some detail. In the case of both *Tenellia pallida* (Alder & Hancock) (EYSTER, 1979) and the ascoglossan *Elysia cauze* (Marcus, 1957) (CLARK *et al.*, 1979) the two developmental patterns appeared to occur within single populations. For *E. cauze* the patterns were separated on a seasonal basis and no laboratory crosses were done. In the case of *T. pallida*, crosses were made by pairing field-collected individuals of unknown reproductive type. All F_1 offspring were not intermediate in character but were of one or the other developmental type in the population. This is similar to the parental type F_1 generation in our study of *Elysia chlorotica*. Unfortunately, in the *T. pallida* study no F_2 generation was produced.

The results of the hybridization of the two *Elysia chlorotica* populations were unexpected. The continuous distribution of capsule length and shell length in the F_2 generation suggests that these characters are controlled by multiple genes. However, this type of distribution is usually expected for the F_1 hybrid generation not the F_2 generation (FALCONER, 1960). Two possible explanations for these results are: (1) the control of certain developmental traits by maternal genes and (2) self-fertilization.

In the case of maternal control it is possible that the morphology of F_1 and F_2 egg capsules depends on the maternal genotype regardless of the genotype of egg and veliger. This model would account for the appearance of Ipswich-type and Martha's Vineyard-type capsules in the F_1 generation because they were deposited by nonhybrid parents, and intermediate or hybrid capsule types in the F_2 generation because they were deposited by hybrid parents. Based on this argument it is more difficult to explain why hatching time is intermediate in the F_2 and not in the F_1 generation. In the case of opisthobranchs, hatching from egg masses is thought to depend on the production of some enzyme by the veliger (DAVIS, 1968). If this is the case, hatching should depend on veliger genotype. However, it is possible that a maternal control of food reserves in egg yolk or capsule albumen, as well as a maternal control of capsule structure, could influence when veligers hatch.

The persistence of parental characters in the F_1 generation also could be explained either by selfing in the parental generation while the F_1 outcrossed or by selfing in both the parental and F_1 generations with an eventual breakdown of parental characters in the F_2 generation. There is one report of self-fertilization in another ascoglossan (KAWAGUTI & YAMASU, 1961). However, because in our laboratory cultures, egg masses laid by isolated individuals developed abnormally, it seems that self-fertilization alone could not explain the maintenance of maternal characters in the F_1 generation.

The production of viable F_1 and F_2 offspring from the crosses does not prove that the two populations are actually one species. More extensive genetic studies would have to be performed to determine whether these populations actually represent one species. However, the results do suggest that crosses between populations could occur in the field and that other populations could exhibit developmental characteristics different from the Ipswich or Martha's Vineyard populations. So far, descriptions of reproductive variation of *Elysia chlorotica* are limited to the two geographically separated populations in this study. It is not known whether populations with the characteristics of the F_1 and F_2 generations of this study occur naturally. Although *E. chlorotica* has a geographic range from Nova Scotia to Florida (MARCUS, 1980), descriptions of development in other populations are limited to one report describing planktotrophic development in Chesapeake Bay and Virginia (VOGEL, 1978). Further studies are underway to identify the extent of variation in other populations and to find whether any correlations exist between certain habitats and development types.

ACKNOWLEDGMENTS

The authors wish to thank Dr. E. E. Webber for assistance in identifying *Vaucheria* species. This work was supported by NIH grant #GM23731 to S. K. Pierce. Additional support was provided by the Intramural Research Program of the National Institute of Neurological and Communicable Diseases and Stroke, NIH. The first author gratefully acknowledges support from the Lerner Foundation for Marine Research of the American Museum of Natural History. This is contribution #198 from the Tallahassee, Sopchoppy and Gulf Coast Marine Biological Association.

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